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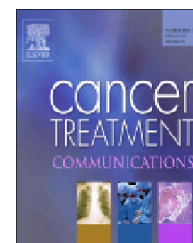


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Targeting TKI-resistance in NSCLC: Importance of rebiopsy and molecular diagnostics—A case study[☆]



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Abstract

We present the case of a 27 year old woman with widely disseminated metastatic adenocarcinoma of the lung who outlived stage IV diagnosis of NSCLC by more than 4 years. Based on rebiopsy and molecular diagnostics of oncogene dependency, appropriately targeted therapies were successfully employed up to a fifth line oral monotherapy with the ErbB family blocker afatinib for a final 11 months. We stress the importance of rebiopsies, the corresponding selection of targeted therapies, and the observed gain in quality of live even in end stage disease.

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1. Introduction

Although well established by now, treatment of lung cancer beyond the first therapy line(s) has only been a development

of the very recent years. In 2004, the ASCO guidelines stated that “there is no current evidence that either confirms or refutes that second-line chemotherapy improves survival in nonresponding or progressing patients with advanced NSCLC.” [1] Meanwhile, with the arrival of tyrosine kinase inhibitors (TKIs) which interfere with the signaling pathway of the epidermal growth factor receptor (EGFR), treatment options have expanded, and even more versatile drugs are about to approach the market. Selected patients have already profited from these drugs and experienced extended treatment options beyond conventional dead ends. The choice of therapy for lung cancer patients who are eligible for treatment with oral EGFR-TKI vs. conventional chemotherapy should be based on evidence from molecular pathology rather than on clinical criteria [2]. However, even though sensitizing mutations in the

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gene coding for EGFR are associated with higher response rates to EGFR-TKI therapies and prolonged progression-free survival, in the end resistant mutants prevail through cellular survival pressure. The most frequent single mutation associated with secondary resistance to first generation EGFR-TKIs such as erlotinib and gefitinib is the point mutation T790M in exon 20 of the EGFR gene, coding for the tyrosine kinase domain and thus extremely sensitive to alterations [3]. The ErbB-family blocker afatinib is a drug that binds covalently to the ATP-binding sites of EGFR (Cys797), HER2 (Cys805) and ErbB4 (Cys 803) [4]. The covalent binding mode allows afatinib greater occupancy of the ATP-binding site than reversible inhibitors, providing the ability to inhibit EGFR T790M in pre-clinical models [4]. Correspondingly, drugs like afatinib may be an alternative for tumors harboring *de novo* or acquired T790M, since an acquired resistance does not make EGFR signaling redundant, as Sequist et al. observed [5].

We report here the case of a young woman with non-small cell lung cancer whose patient history showed a typical series of mutations, and how her therapy was tailored based on molecular evidence.

2. Case presentation

The patient was first diagnosed with non-small cell lung cancer in May 2007, at 27 years of age. The initial PET-CT showed a 3 cm tumor in the right middle lobe with connection to the pleura and pleural effusion on this site of the lung. No affected lymph nodes were detected. However, the tumor had already spread to the bone and metastases were detected in the spine. A TTF-1 positive adenocarcinoma of the lung was diagnosed by CT guided biopsy of the mass in the lung. The pleural effusion also contained malignant cells of a TTF-1 positive adenocarcinoma. In addition, a suspected breast cancer was in fact verified as another metastasis of the lung tumor after biopsy and histological analysis of a mass in the left breast.

This young woman had never smoked, nor was there any evidence of cancer in the family history, yet the tumor had already metastasized beyond curative treatment options at the time of diagnosis.

As first-line palliative chemotherapy she received paclitaxel and carboplatin for 6 months, resulting in a partial remission lasting for over 3 months. From January to February 2008 she was treated with pemetrexed, but the tumor progressed further. At this point molecular analysis of the initial tumor sample identified an activating mutation in exon 19 of the gene coding for the EGF receptor (Fig. 1A), but no other relevant mutations within the EGFR gene were identified in that Sanger analysis.

Erlotinib was started in February 2008 and continued until December 2009, when, after 22 months, the disease progressed again and a tumor re-biopsy was taken. Besides the activating mutation in exon 19 of the EGFR gene it now revealed a secondary T790M point mutation in exon 20, conferring resistance to erlotinib. Sanger Sequencing results of this analysis are presented in Fig. 1B.

Correspondingly, in January 2010 another palliative chemotherapy consisting of gemcitabine and cisplatin was initiated. Due to neurotoxicity (tinnitus), the platinum component was switched after 1 month to carboplatin, which was

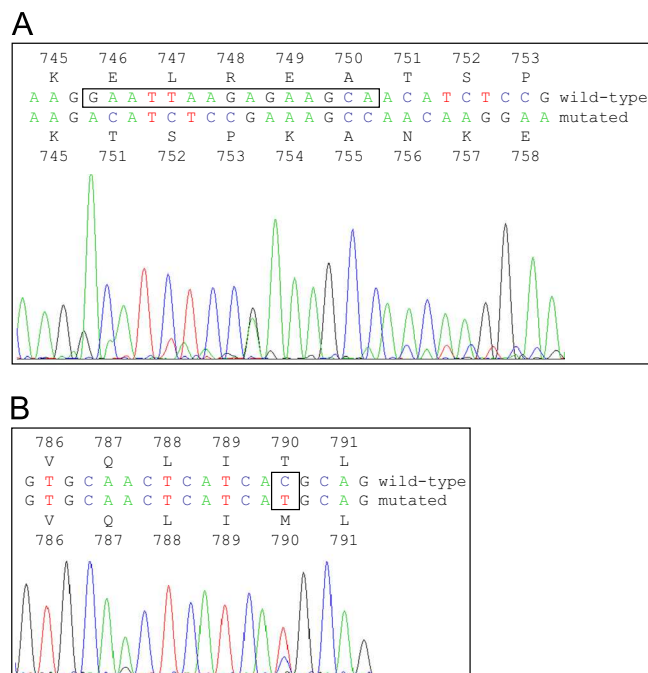


Fig. 1 (A) Detail of the forward sequencing reaction of exon 19 showing the deletion c.2236_2250del leading to p.E746_A750del. (B) Detail of the forward sequencing reaction of exon 20 showing the point mutation c.2369C>T leading to p.T790M. Mutated nucleotides are marked by open boxes.

better tolerated and kept up for 4 months. Best tumor response was partial remission, and the regimen was finished as planned in May 2010.

In July 2010, the patient experienced severe thoracic pain, shortness of breath, and analgesia refractory headaches. Complete tumor restaging including MRI and CT imaging was performed. Besides the dissemination in lung, there was a pericardial effusion that had to be drained and treated by pericardial instillation of mitoxantrone. Both metastases in the thoracic vertebrae had not progressed, nor had the tumor grown at or near the primary sites. However, the headaches had been caused by disseminated brain metastases, which were treated with palliative 2D irradiation of the whole brain with a total dose of 30 Gy.

Based on the molecular histopathology with the activating mutation in exon 19 and the T790M mutation, the patient was subsequently included into a compassionate use program of the ErbB-family blocker afatinib, starting on 50 mg once daily. Due to diarrhea, the dose was reduced after 4 weeks to 40 mg, which was tolerated very well, the only side effect being mild rash (grade 1).

Further tumor stagings were performed 2 months and 5 months after start of the oral fifth-line therapy with afatinib and confirmed stable disease. The patient herself reported feeling well, had no complaints, felt full of energy, and was even able to spend and enjoy holidays.

By the end of June 2011, however, 11 months after starting afatinib, another clinical progression occurred. There were pleural effusions again and axillary lymph nodes were palpable. From those lymph nodes, new samples were taken and analyzed. The analyses confirmed the earlier results, revealing both T790M in exon 20 of the EGFR gene

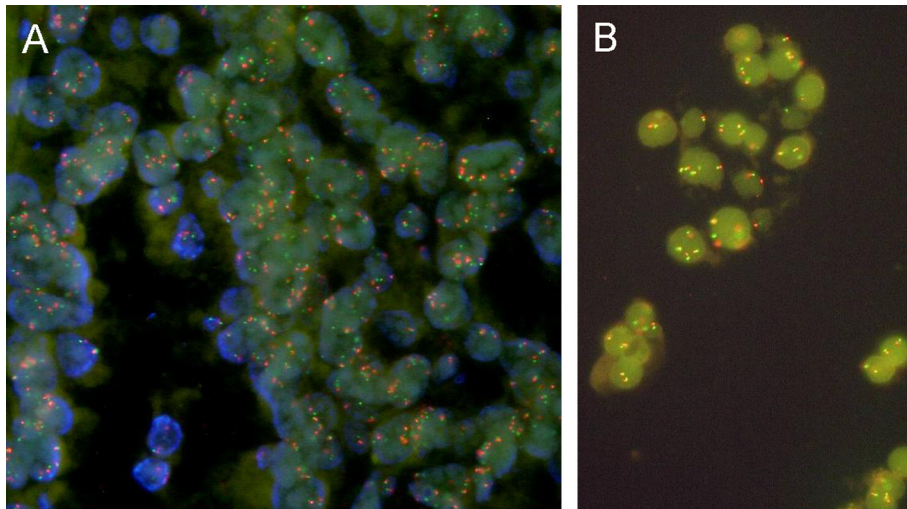


Fig. 2 (A) FISH analysis of the lymph node metastasis after fifth-line oral therapy. By counting 60 tumor cell nuclei, a ratio of 226 c-Met signals (green) to 222 chromosome 7 copies (CEN7, orange) was found with 50% of tumor cells harboring ≥ 4 c-Met copies. According to the Colorado scoring system which is also applicable to c-Met, this result was considered positive (“high polysomy”). Re-analysis of a preserved previous tumor sample confirmed that c-Met had not been amplified before. (B) Previous tumor sample without relevant increase in c-Met gene copies: 187 c-Met and 187 CEN7 copies were counted in 60 tumor cells (cell block of a previous cytology sample) and that this new genetic lesion had occurred in parallel to the clinical resistance to afatinib. All other oncogenes assessed (K-RAS, B-RAF, PIK3CA) were still wild type. The patient died 4 weeks later from respiratory complications. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and a deletion which has been associated with increased sensitivity to EGFR-TKI [6] in exon 19 of the same gene. Fluorescence in situ hybridization (FISH)-testing revealed amplification of the oncogene c-Met (Fig. 2). In contrast, retrospective analyses of the former tumor samples did not show an amplification of the oncogene c-Met.

3. Discussion

This case shows some practical evidence for mutation development in a series. First, there was the primary sensitizing mutation on exon 19, indicating susceptibility to EGFR-TKI. Then, following treatment with EGFR-TKI, secondary resistance resulted from development of the point mutation T790M, but the tumor was still sensitive to treatment with afatinib. Over time, amplification of c-Met led to tertiary and final resistance. Unfortunately clinical deterioration did not allow starting additional treatment with c-Met inhibitors.

Molecular analyses of the tumor showed that at every point in time the clinical response of the tumor to EGFR inhibitors could be explained by the results of the genetic analyses. Treatment decisions that are based on molecular evidence result in the respective cellular and correspondingly also clinical responses. Therefore, both biopsies and re-biopsies are essential. Although the role of T790M as a negative predictive marker for first generation EGFR-TKI is controversial [7] there is some evidence that patients with *de novo* T790M profit less from erlotinib treatment and have a significantly shorter progression free survival than T790 wild type patients [8,9]. Thus early molecular screening for T790M might help to choose drugs that are specifically effective even when a T790M mutation is present.

Direct binding assays showed that in T790M not steric blockade but increased ATP affinity is the primary mechanism for resistance against ATP-competitive kinase inhibitors [10]. Correspondingly, reversible inhibitors such as erlotinib or gefitinib retain some but very limited efficacy in T790M mutants, whereas irreversible inhibitors like afatinib have been shown to overcome this resistance through covalent binding in preclinical assays [11]. Despite the poor in vitro activity of reversible TKIs against T790M mutants some clinical evidence exists that reintroduction of a first generation EGFR-TKI like erlotinib may again be effective after occurrence of a resistance mutation, even independently of intermittent chemotherapy [12]. Evidence based data for this situation are still limited and in the absence of phase 3 studies retrospective papers report results only for low patient numbers [12]. However, as retreatment efficacy has been documented even for erlotinib and gefitinib, we hypothesized that an experimental treatment employing an EGFR-TKI with added efficacy such as afatinib might be a very promising approach, and that further clinical investigations are warranted to clarify the role of irreversible EGFR-TKIs in T790M mutated NSCLC.

Besides the T790M mutation, upregulation of HER2 has been shown by Takezawa et al. to be another mechanism of resistance to reversible EGFR-TKIs [13]. HER2 status was not determined in our patient, but according to the findings of Takezawa et al. it should be considered that the efficacy of afatinib after failure of the reversible EGFR-TKI may also be in part due to its ability to irreversibly block the entire ErbB family which also includes HER2 [4]. Although T790M, as a secondary mutation after initial EGFR-TKI success, is considered a mutation with less impact on the remaining overall survival time than some other resistance mechanisms e.g. cMET amplification [14], progressive disease still occurs and is associated with more tumor lesions, patient

complaints and impaired quality of life. All in all, treatment benefits may or may not include additional life time gain, but what impressed us most was the gain in quality of life our patient experienced. This observation is supported by results of the Phase IIb/III LUX-Lung 1 trial which investigated afatinib versus placebo in patients with advanced, metastatic lung adenocarcinoma after failure of one or two lines of chemotherapy and reversible EGFR TKI [15]. The LUX-Lung 1 study was negative for its primary endpoint (overall survival: HR 1.08, $p=0.74$). However, patients in the afatinib arm experienced a significant delay in disease progression (median PFS was 3.3 month for afatinib vs. 1.1 month for placebo; HR 0.38, $p<0.0001$) and significant improvements in NSCLC-related symptoms (cough, dyspnea, and pain), global quality of life, and physical functioning [15,16]. In the case presented here our patient was free of pain and side effects, she felt full of energy and was able to interact with her family and friends without restrictions for the whole treatment duration with afatinib over 11 months. She was even able to go on holidays despite having end stage lung cancer.

4. Conclusion

The palliative treatment with afatinib was definitely a success, lasting for almost another year.

It is also worth to mention that this treatment success in fifth line was not achieved by multiple drug combinations but using a simple oral monotherapy which was administered by the patient herself, at home.

Despite their invasive nature we believe that it is highly important and warranted to take rebiopsies at the time of progression in NSCLC. Since the molecular profile may change under targeted therapy and the number of new molecular targeted therapeutic options is rising, we believe that the possibility of a new treatment prevails the risk of an invasive rebiopsy. It is a major challenge of the future that we strive to convince our patients of the need to have rebiopsies taken in the appropriate situations.

All in all, our case critically underlines the value of rebiopsies and timely molecular diagnostics to deliver appropriate therapies after development of resistance to targeted therapies with EGFR-TKIs.

Conflict of interest statement

None of the authors report any financial or personal relationship that bias this manuscript including employments, stock ownership, patent applications or other relevant funding. Jan Stoehlmacher-Williams has served as advisory board member receiving compensation and has received honoraria for speeches from Boehringer Ingelheim.

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Consent statement

Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

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